Role of endothelin in progressive proteinuric nephropathies

CHAPTER 8

Summary

Experimental and human proteinuric progressive nephropathies are associated with tubulo-interstitial injury whose pathogenesis is still undefined. Studies have established that an excessive and sustained protein traffic through the nephron, as a consequence of high glomerular capillary pressure, is invariably associated with the progress of the disease. Exuberant protein excretion induces forced reabsorption by proximal tubular cell, that changes its phenotype overexpressing vasoactive and inflammatory molecules. Release of such molecules towards the interstitium triggers an interstitial inflammatory reaction that precedes renal scarring. Among mediators challenged by protein overload is endothelin-1 (ET-1), a peptide with vasoconstrictor and proliferative properties, which, by virtue of its chemotactic activity, attracts blood monocytes and stimulates them to produce cytokine, which would further amplify the interstitial inflammatory reaction. These features together the bulk of evidence that ET-1 renal gene expression and synthesis are increased in proteinuric progressive nephropathies has stimulated research on ET-1 and on endothelin receptor antagonists, as novel therapeutic tools to slow renal disease progression (reviewed in **chapter 1**).
In this thesis the definition of kidney cells overexpressing in vivo ET-1 during the course of the experimental renal disease is accomplished. Furthermore, attempts to slow progression of renal disease in progressive renal injury with endothelin receptor antagonists alone or in combination with angiotensin converting enzyme inhibitors are pursued. Finally, the possible contribution of ET-1 to the development of interstitial fibrosis and inflammation in renal transplant patients chronically receiving CsA is addressed.

In **chapter 2**, we first evaluated the cellular origin of excessive renal ET-1 production in the renal mass reduction model and then related ET-1 distribution to the development of kidney lesions. Animals with renal mass reduction and control rats were studied early after surgery (day 7,14,21,28). Renal mass reduction rats developed proteinuria accompained first by tubulo-interstitial changes and subsequently by glomerulosclerosis. A parallel increase of renal endothelin-1 gene expression, evaluated by nonradioactive in situ hybridization with a rat ET-1 riboprobe, and synthesis of the corresponding peptide assessed as urinary ET- 1 excretion was observed in renal mass reduction rats versus controls from day 14 consistent with the distribution of tubulo-interstitial lesions. At day 14, overexpression of ET-1 mRNA staining was mainly localized to the cytoplasm of tubular cells, whereas glomeruli were negative. At day 28, ET-1 expression further increased in renal mass reduction rats as compared with controls, and the staining was evident also in glomeruli. Thus, in renal mass reduction, a progressive up-regulation of ET-1 occurs during the development of renal injury, that first involves the tubules and, only in a subsequent phase, the glomeruli.

In **chapter 3**, we first studied renal ET_A and ET_B receptor gene expression in rats with remnant kidney on days 7, 30 and 120 after the surgical procedure. While renal expression of ET_A was unaffected, ET_B receptor gene was significantly up-regulated with time in rats with remnant kidney, being 3.5-fold and sixfold higher than sham-operated rats at days 30 and 120. We also evaluated whether bosentan, a nonpeptidic ET_A and ET_B receptor antagonist, offered better protection against renal disease progression than reported for ET_A-selective blockers and whether it improved survival in animals with renal ablation. Rats with renal mass reduction were given bosentan orally or its vehicle (carboxymethyl cellulose) from day 7 after surgery and were followed until the death of the vehicle-treated animals. Bosentan only partially prevented the increases in blood pressure and proteinuria, but had a remarkable protective effect on renal function and significantly prolonged animal survival, suggesting that blocking both renal ET_A and ET_B receptors might have implications in the treatment of human progressive nephropathies.

In **chapter 4**, we investigated whether an unselective ET_A/ET_B receptor antagonist, PD 142,893, was renoprotective when given to streptozotocin diabetic rats when animals were already proteinuric. The effect of PD 142,893 was compared with that of an ACE inhibitor, lisinopril, known to retard progressive renal disease in experimental and human diabetes. PD 142,893 normalized systemic blood pressure, reduced urinary protein and albumin excretion, and ameliorated renal blood flow in diabetic rats, but it did not affect such parameters in control rats. Lisinopril had a renoprotective effect comparable to PD 142,893, although a better control of systemic blood pressure was achieved. Northern blot analysis of ET-1 mRNA revealed upregulation of ET-1 gene in the diabetic kidney. Similar results were obtained by non radioactive in situ hybridization in glomeruli and tubuli of diabetic rats. Both treatments remarkably attenuated exaggerated renal ET-1 gene expression. These data suggest that ET-1 is a contributory mediator of kidney damage in diabetes and indicate that ET receptor antagonists may represent a new therapeutic mean for treatment of progressive diabetic nephropathy.

In **chapter 5**, we compared the effect of an ET_A receptor antagonist and an ACE-inhibitor given as single therapies with a combination of the two drugs in rats with accelearated passive Heymann nephritis, which mimics advanced phases of human membranous nephropathy. Animals were treated from day 7 to 8 months with the ET_A receptor antagonist LU-135252 or the ACE-inhibitor trandolapril or the combination of the two. Either LU-135252 or trandolapril given alone prevented the increase in systolic blood pressure. Com-

bined therapy was even more effective than single drugs. While LU-135252 and trandolapril reduced proteinuria by 23 to 25% the drug combination resulted in 45% lowering of urinary proteins. Serum creatinine was significantly decreased by the combination, but not by the single drugs. Glomerulosclerosis and tubulointerstitial damage were more reduced by combined therapy than by LU-135252 or trandolapril alone. These data suggest that contemporary blocking angiotensin II (AII) and ET-1 in an accelerated model of PHN had an additive renoprotective effect than single blocking AII or ET-1 and would represent a therapeutic advantage for renal disease patients who do not completely respond to ACE inhibitors.

In **chapter 6**, we addressed the issue of the cellular origin and mediators of tubulointerstitial changes after chronic CsA administration in kidney transplant patients. As a part of a clinical trial in kidney transplant recipients on triple immunosuppressive therapy (CsA, azathioprine and steroid) which includes renal biopsy as "per protocol", 22 patients enrolled between 12 and 24 months post-transplantation underwent renal hemodynamic evaluation by measuring glomerular filtration rate and renal plasma flow by the plasma clearance of unlabeled iohexol and the renal clearance of para-aminohippuric acid, respectively. In parallel, the CsA pharmacokinetic profile was also determined. A week later, a protocol biopsy of kidney graft was performed. Light microscopy examination and localization of endothelin-1, and two chemokines, RANTES and monocyte chemoattractant protein-1 gene expression by in situ hybridization in the graft specimens were evaluated and related to the pattern of histologic lesions. Ten out of 22 kidney transplant recipients who underwent the protocol biopsy had CsA nephrotoxicity, eight had chronic rejection, and four had no lesions at histological examination. The total daily exposure to CsA was higher in patients with CsA nephrotoxicity than in those with chronic rejection or no lesions at biopsy. Renal function was preserved in the CsA toxicity group as compared with the chronic rejection group, despite some degree of renal hypoperfusion. Tubular atrophy and striped interstitial fibrosis were found in all patients with light microscopical evidence of CsA nephrotoxicity, whereas glomerular and arteriolar lesions were less frequent. Intense staining for endothelin-1, RANTES, and monocyte chemoattractant protein-1 mRNAs selectively localized at tubular epithelial cells was found in biopsies taken from patients with CsA nephrotoxicity, but not in the chronic graft rejection group, whose tubuli had only minimal staining for RANTES mRNA on a few occasions.
Long-term CsA administration to kidney allograft recipients leads to tubulointerstitial injury independently of its vascular effect. The possible contribution to the development of interstitial fibrosis of inflammatory and growth factors released by tubular cells in which CsA accumulates is proposed.